

X-Ray Diffraction Studies of Addition Compounds of Amylose with Inorganic Salts*

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Although granular starch adsorbs many salts from aqueous solution,¹ the adsorption complexes do not yield x-ray diffraction patterns characteristic of the complex. At low concentrations of salt, the starch generally retains its typical diffraction pattern; if the concentration is high and the starch granules are swollen, an amorphous x-ray pattern results. The diffraction method thus gives little information on the specific interaction of salts with granular starch. In a previous investigation² of oriented alkali amylose filaments, it was found that several salts in aqueous alcohol solutions could be exchanged for the alkali. Especially good diffraction patterns were produced by amylose addition compounds formed by this method with a series of potassium salts. Because these compounds appeared to be isomorphous and possess high symmetry, an attempt has been made to analyze their diffraction patterns in some detail.

EXPERIMENTAL WORK AND RESULTS

Alkali amylose was prepared² by deacetylating oriented filaments of amylose triacetate at 25°C. in 0.1 to 0.2 *N* potassium hydroxide in 75% ethanol. To make the amylose compounds by ion exchange, filaments of alkali amylose were held taut in stainless steel clamps and immersed in solutions of the appropriate salts. In most cases, the solvent was 75 or 80% ethanol. At lower concentrations of ethanol the addition compounds were unstable, whereas at higher concentrations the solubility of the salts was reduced, and the rate of exchange was slow. The exchange reaction was judged complete when phenolphthalein solution placed on the filaments remained colorless. For determination of composition, the filaments were allowed to equilibrate with the salt solution for two weeks. Addition compounds of amylose with the iodide, bromide, acetate, formate, and propionate of potassium and with sodium bromide and ammonium fluoride were prepared by the exchange method. To prepare potassium bicarbonate amylose, the alkali in the filaments was neutralized with gaseous carbon dioxide. This reaction was rapid when carried out in a moist atmosphere.

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Composition. On removal from the salt solutions, the filaments were wiped dry with absorbent tissue. Before analysis, alcohol was removed from the filaments by humidification in a saturated atmosphere of water vapor. Because the addition compounds were prepared in small amounts, microanalytical procedures were used for determining composition. Vacuum-dried filaments of potassium iodide and potassium bromide amylose were analyzed for halogen by the Carius method. Filaments of the other potassium compounds, were ashed, and potassium was determined as the sulfate.

The compositions of the potassium iodide and the potassium acetate addition compounds as a function of the concentration of the respective salt in the aqueous alcohol exchange medium was determined. Figure 1 presents the data on filaments equilibrated with four concentrations of potassium iodide in 75% ethanol. Absorption of salt increased rapidly with concentration of salt in the exchange medium up to a concentration of 8%;

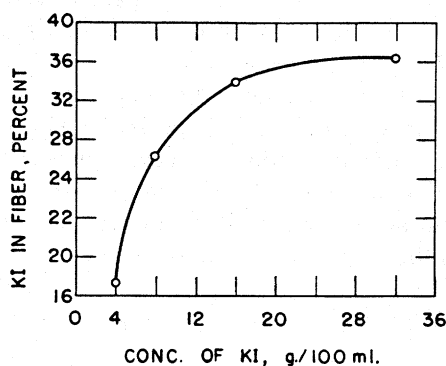


Fig. 1. Composition of potassium iodide amylose filaments as a function of the concentration of potassium iodide in 75% ethanol solutions.

above this concentration, the slope of the curve decreases rapidly, and becomes nearly independent of concentration as saturation of the solution is approached. The maximum content of potassium iodide in the filaments was 36%, which is in reasonable agreement with the potassium iodide content, 33.9%, required by the formula $1\text{KI} \cdot 2\text{C}_6\text{H}_{10}\text{O}_5$. Filaments equilibrated with saturated potassium bromide in 75% ethanol contained 25% salt, a composition also in good agreement with the value 26.9%, demanded by the formula $1\text{KBr} \cdot 2\text{C}_6\text{H}_{10}\text{O}_5$. Filaments equilibrated with 4% potassium formate in 80% methanol contained 19% salt, whereas the theoretical value for $1\text{K formate} \cdot 2\text{C}_6\text{H}_{10}\text{O}_5$ is 20.6%. As described below, diffraction patterns of potassium bromide, potassium formate and potassium iodide amylose indicate that these compounds are isomorphous and possess tetragonal structures.

The composition curve for potassium acetate amylose, shown in Figure 2, exhibits a marked break at a salt content of about 25%. This suggests

compound formation according to the formula $1\text{KAc} \cdot 2\text{C}_6\text{H}_{10}\text{O}_5$, which requires 23.3% potassium acetate. Diffraction patterns showed that the compound has a tetragonal structure, with unit cell dimensions close to those of the iodide, bromide, and formate addition compounds. In contrast to the iodide and bromide, the maximum absorption of potassium acetate by the filaments was not reached at the ratio of one salt molecule to two glucose residues, but attained a value of 42% in a saturated solution. This is slightly greater than the content required by the formula $1\text{KAc} \cdot 1\text{C}_6\text{H}_{10}\text{O}_5$. Diffraction patterns of filaments having this composition showed a new structure with an orthorhombic unit cell. Filaments equilibrated with 15% potassium propionate in 80% ethanol contained 34%

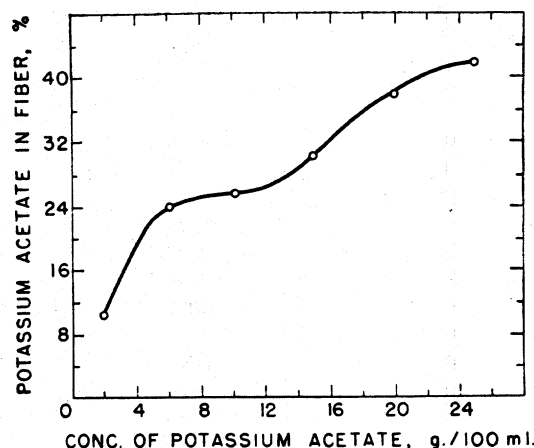


Fig. 2. Composition of potassium acetate amylose filaments as a function of the concentration of potassium acetate in 80% ethanol solutions.

salt, corresponding to a 1:1 ratio of salt to glucose residues. These filaments were isomorphous with potassium acetate amylose of similar composition.

The composition of potassium bicarbonate amylose filaments was determined by the amount of potassium hydroxide in the filaments exposed to moist carbon dioxide. Two samples of potassium hydroxide amylose having 11 and 15% alkali, respectively, and having the approximate composition $1\text{KOH} \cdot 2\text{C}_6\text{H}_{10}\text{O}_5$, were allowed to react with carbon dioxide. In both cases, there was complete conversion of the alkali amylose structure to the tetragonal structure, presumably resulting from the formation of the potassium bicarbonate addition compound. Weak powder lines, characteristic of crystalline potassium bicarbonate, appeared on the diffraction patterns of both samples. In deducing structures from diffraction and composition data, it is assumed that no substantial error is made by taking the composition of crystalline and noncrystalline parts of the filaments to be alike.

TABLE I
CALCULATED AND OBSERVED SIN θ VALUES FOR POTASSIUM BROMIDE
AMYLOSE

<i>hkl</i>	Sin θ (obs.)	Sin θ (calcd.)	I (est.)	<i>hkl</i>	Sin θ (obs.)	Sin θ (calcd.)	I (est.)
010	0.0724	0.0719	W ⁻	013	0.1609	0.1604	W ⁻
110	.1014	.1016	W ⁻	113	.1764	.1757	M ⁻
020	.1439	.1437	W ⁻	023	.2028	.2030	W ⁻
120	.1608	.1607	W ⁺	123	.2147	.2153	S ⁺
220	.2029	.2032	W	223	.2481	.2487	W ⁻
130	.2269	.2272	W ⁻	033	.2587	.2589	W ⁻
230	.2589	.2590	M	133	.2684	.2687	M
040	.2877	.2874	W ⁻	233	.2958	.2961	W
140	.2963	.2968	M ⁻	143	.3297	.3291	W ⁻
240	.3210	.3213	W ⁻	333	.3367	.3369	W ⁻
150	.3672	.3664	W=diff.	243	.3519	.3519	W
450	.4603	.4600	W=diff.	053		.3869	
				343	.3909		W diff.
011	.0866	.0863	W ⁻	153		.3934	
111	.1126	.1123	W ⁻	253	.4118	.4126	W ⁻
021	.1516	.1514	M ⁻				
121	.1674	.1676	S	014	.2044	.2042	W
221	.2084	.2088	M ⁻	124	.2495	.2497	W ⁻
031	.2202	.2208	M	224	.2796	.2790	M ⁺
231	.2637	.2634	W ⁻	134	.2970	.2969	W ⁻
041	.2907	.2913	W	234	.3217	.3220	W ⁺
141	.2998	.3008	W ⁺	144	.3523	.3526	W
331	.3082	.3086	W ⁻	334	.3608	.3598	W ⁻
241	.3252	.3248	W ⁺	244	.3730	.3739	W
341				344			
501	.3633	.3624	W	054	.4075	.4070	W diff.
511	.3697	.3695	W	164	.4760	.4770	W ⁻
251	.3899	.3899	W ⁻	264		.4930	
351	.4212	.4217	W diff.	454	.4962	.4982	W diff.
061	.4344	.4337	W diff.				
012	.1199	.1196	W	015	.2503	.2495	W ⁺
112	.1396	.1395	M ⁺	025	.2784	.2789	W ⁻
022	.1725	.1726	W	125	.2885	.2880	M
122	.1867	.1869	S	035	.3213	.3218	W ⁺
032	.2356	.2358	M ⁻	135	.3297	.3297	W ⁺
132	.2462	.2465	M	235	.3517	.3524	W
232	.2759	.2761	W ⁻	145		.3806	
042	.3026	.3029	W	335	.3840	.3873	W ⁻
142	.3121	.3113	W ⁻ diff.	245	.4002	.4004	W ⁻
242	.3352	.3352	W				
052				016	.2959	.2956	W ⁺
342	.3727	.3718	W	026	.3208	.3208	W ⁺
152	.3797	.3786	W ⁻	136	.3670	.3658	W
252	.3993	.3986	W ⁻	236	.3850	.3852	W ⁻
442	.4178	.4175	W	146		.4123	
352	.4298	.4297	W	336	.4158	.4185	W diff.
062	.4419	.4416	W ⁻	246	.4303	.4307	W ⁻
262	.4651	.4644	W ⁻				
362	.4924	.4914	W ⁻				

X-Ray Diffraction Patterns. Patterns for indexing were obtained in a cylindrical cassette, with filtered $\text{CuK}\alpha$ radiation. Representative patterns of potassium bromide amylose and potassium acetate amylose are shown in Figures 3 and 4. Filaments mounted with the fiber axis perpendicular to the beam showed no reflections from the fiber repeat period. To observe these reflections, filaments were oscillated with the fiber axis inclined to the beam. Orders of the fiber repeat period out to the sixteenth were recorded. Intensities were estimated visually by comparison with a

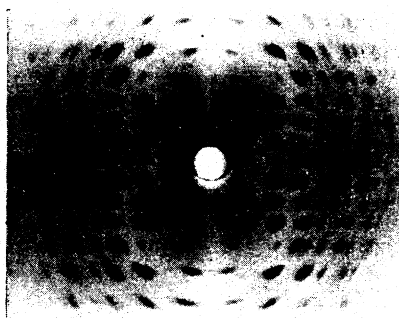


Fig. 3. Potassium bromide amylose (tetragonal structure).

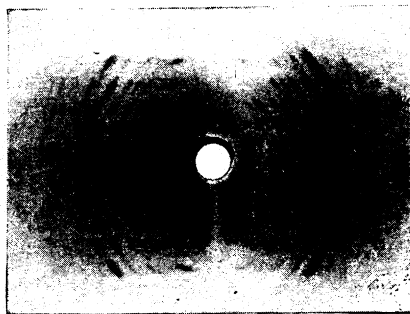


Fig. 4. Potassium acetate amylose (orthorhombic structure).

scale of known relative intensities. Absorption effects in the halogen-containing filaments were minimized by selecting samples less than 0.1 mm. thick.

Unit Cells. Filaments of all the compounds gave well-oriented diffraction patterns, and the unit cell dimension corresponding to the fiber repeat period was determined from layer line separations. Lateral cell dimensions were determined by conventional graphic methods. Table I shows observed $\sin \theta$ values and values calculated for a tetragonal unit cell having $a_0 = 10.7$ Å. and $c_0 = 16.1$ Å. for potassium bromide amylose. The good agreement for the eighty-three forms observed on six layer lines gives considerable confidence in the unit cell selected. Tables II, III, and IV list the cell dimensions of all compounds for which a complete unit cell determination was made.

In Table II are listed the cell dimensions for filaments exposed to room conditions and having a water content of about 10%, which corresponds to one molecule of water per glucose residue. The salt content of these filaments was approximately that required by the formula $1\text{KA} \cdot 2\text{C}_6\text{H}_{10}\text{O}_6$. All five compounds have tetragonal unit cells with nearly the same dimensions. Removal of water by vacuum drying for 3 days at 80°C . resulted in a change (generally a decrease) in unit cell dimensions, as shown in Table III. Orthorhombic cell dimensions of the potassium acetate and potassium propionate addition compounds having composition approximating the formula $1\text{KA} \cdot 1\text{C}_6\text{H}_{10}\text{O}_6$ are listed in Table IV. To compute

TABLE II
TETRAGONAL UNIT CELL DIMENSIONS OF HYDRATED AMYLOSE ADDITION COMPOUNDS^a

Amylose compound with	$a_0 = b_0$ A.	c_0 (fiber axis), A.
KBr	10.7	16.1
KI	10.7	16.1
K formate	10.8	16.1
K acetate	10.8	16.1
K bicarbonate	10.8	15.8

^a Filaments equilibrated with the atmosphere of the room and containing about 10% moisture.

TABLE III
TETRAGONAL UNIT CELL DIMENSIONS OF ANHYDROUS AMYLOSE ADDITION COMPOUNDS^a

Amylose compound with	$a_0 = b_0$ A.	c_0 (fiber axis), A.
KBr	10.2	16.4
KI	10.2	16.4
K formate	10.5	15.9

^a Filaments vacuum-dried at 80° for 3 days and sealed in thin-walled glass capillaries.

TABLE IV
ORTHORHOMBIC UNIT CELL DIMENSIONS OF AMYLOSE ADDITION COMPOUNDS^a

Amylose compound with	a_0 , A.	b_0 , A.	c_0 (fiber axis), A.
K acetate	11.0	18.1	17.9
K propionate	11.4	18.0	17.6

^a Filaments equilibrated with the atmosphere of the room.

TABLE V
COMPOSITION AND DENSITY OF AMYLOSE ADDITION COMPOUNDS

Amylose compound with	Salt content, %	Water content, %	Density
KI	29	11.2	1.84
KI	33	0.0	1.79
KBr	22	10.6	1.67
KBr	25	0.0	1.63
K formate	18	7.5	1.53
K formate	19	0.0	1.52
K acetate	20	7.6	1.53
K acetate	22	0.0	1.52

the number of glucose residues in the unit cell, it is necessary to know the density of the filaments. Densities were determined by flotation in carbon tetrachloride-hexane-methylene iodide mixtures; the results are recorded in Table V. Computation of Z , the number of residues per unit cell, by the formula $Z = [abc/(162 \times 1.65)]\rho f$, where ρ is the density and f the fraction of carbohydrate material in the sample, indicates integral value eight for all the tetragonal structures.

Space Group of Tetragonal Structures. Successful indexing of a fiber pattern on the basis of a lattice with equal lateral identity periods does not necessarily require that the symmetry of the lattice be tetragonal. The true symmetry may be orthorhombic or even lower, the orthogonality and equality of the lateral identity periods being fortuitous. In the present case, however, strong support for assignment of tetragonal symmetry is provided by the observation that both hydrated and anhydrous addition compounds can be indexed on tetragonal lattices. Symmetrical shrinkage in the a and b directions, which occurred on the removal of water, indicates the equivalence of these directions in both the hydrated and anhydrous structures. Further evidence is provided by the appearance of only those

($00l$) reflections for which $l = 4n$ out to $n = 5$. It is therefore probable that the structure has a fourfold screw axis parallel to the fiber axis, and this requires that the symmetry be tetragonal or higher.

The assumption of a tetragonal lattice with four fold screw symmetry, and the requirement that the optically active amylose molecules cannot be related by planes of symmetry, limit the possible space groups to those isomorphous with the point groups C_4 and D_4 . For all the tetragonal compounds, reflections from the forms ($h00$) and ($0k0$) were observed only for $h = k = 2n$, and no systematic extinctions occurred in the general forms



Fig. 5. Patterson projection $P_w(u,v)$ for potassium bromide amylose. Predicted positions of peaks from Br—Br vectors are designated by +, K—K vectors by \times , and Br—K vectors by \circ .

(hkl), further restricting the probable space groups to the enantiomorphic pair $P_{4,2,1}$ and $P_{4,2,1}$. As discussed below, if the symmetrical *trans* ring conformation of minimum internal steric hindrance is accepted as the best model for the alpha-D glucose residues, the most probable space group is $P_{4,2,1}$. Insufficient equatorial reflections established systematic extinctions in the forms ($h00$) and ($0k0$) beyond doubt, and therefore, space groups $P_{4,1}$ and P_4 are not rigorously excluded. Long exposures of potassium bromide amylose, however, have brought out only the following weak reflec-

tions (not reported in Table I): 530, 610, 910, 930, 710, 720, 660, and 830. Failure of odd orders of ($h00$) or ($0k0$) to appear supports the selection of $P_{4_2,2_1}$ or $P_{4_1,2_1}$ as the most probable space group.

Patterson Projections. The high scattering power of iodine and bromine should make it possible to locate these ions from Patterson projections of the corresponding addition compounds. Because the best intensity data were available for potassium bromide amylose, Patterson projections ($P_w(u,v)$ and $P_v(u,w)$) were computed for this compound; and the results are presented in Figures 5 and 6, respectively. Table VI gives observed intensities and F^2 values for the forms contributing to these projections.

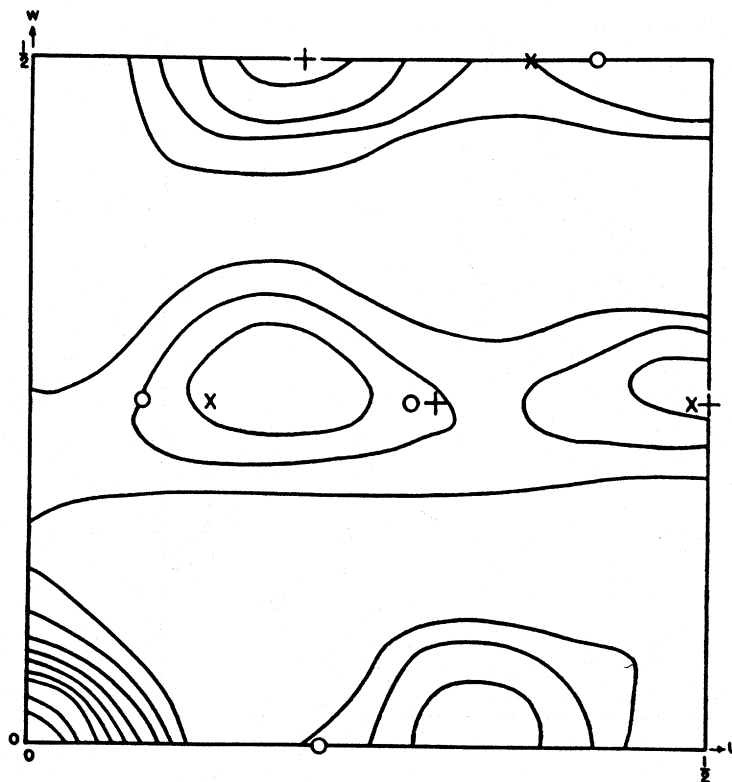


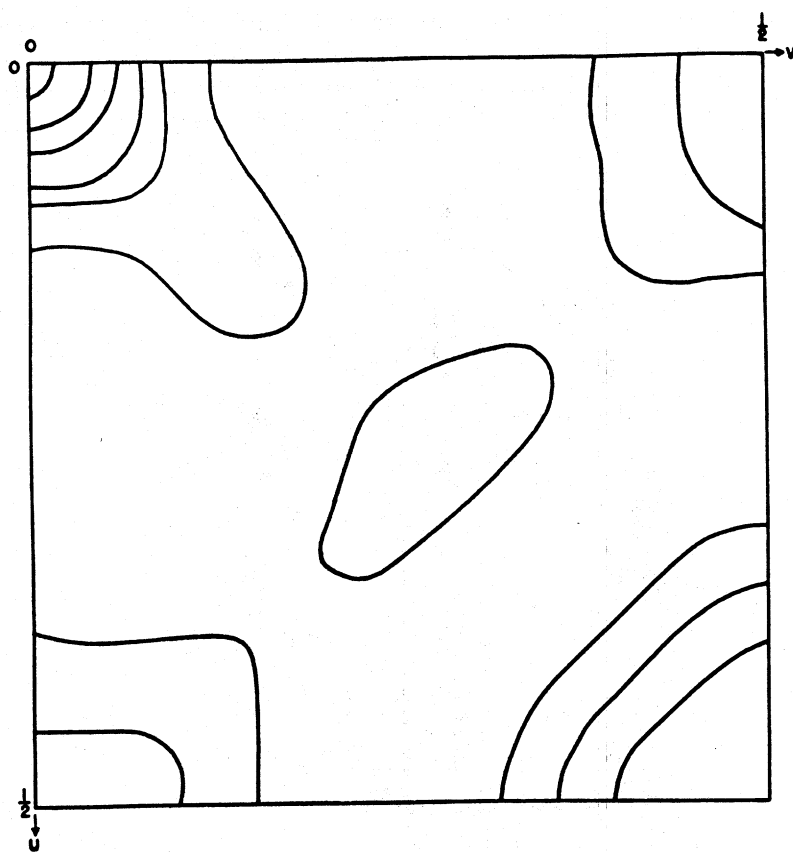
Fig. 6. Patterson projection $P_v(u,w)$ for potassium bromide amylose. Predicted positions of peaks from Br—Br vectors are designated by +, K—K vectors by x, and Br—K vectors by o.

From the composition, density, and unit cell dimensions, it is concluded that there are four molecules of potassium bromide per unit cell. Potassium and bromide ions must therefore occupy fourfold positions in either space group $P_{4_2,2_1}$ or $P_{4_1,2_1}$. A further requirement is that the distance between the potassium and bromide ions should equal the sum of their ionic radii, 3.28 Å. With these restrictions, the major peaks on the Patterson projections can be assigned to Br[−]—Br[−], K⁺—K⁺, and K⁺—Br[−] interactions

TABLE VI

OBSERVED INTENSITIES AND F^2 VALUES FOR POTASSIUM BROMIDE AMYLOSE

$hk0$	$I_{\text{obs.}}$	F^2_{hk0}	$h0l$	$I_{\text{obs.}}$	F^2_{h0l}	$h0l$	$I_{\text{obs.}}$	F^2_{h0l}
110	9	23	101	16	28	207	3	13
020	—	—	102	8	13	208	2	8
120	20	42	103	7	12	209	2	9
220	10	54	104	8	13	301	30	174
130	6	18	105	25	43	302	18	105
230	38	136	106	13	23	303	7	42
040	8	65	107	15	29	305	9	60
140	32	134	109	7	14	400	3	24
240	12	56	201	26	97	401	6	43
430	2	22	202	13	49	402	3	26
150	6	33	203	8	29	004	—	300
540	8	62	205	6	24			

Fig. 7. Patterson projection $P_v(u,v)$ for potassium formate amylose.

by placing the potassium ions and bromide ions at $(19/60, 19/60, 0)$ and $(6/60, 6/60, 0)$, respectively, and positions derived by symmetry.

Positions of computed and observed maxima are indicated in Figures 5

TABLE VII
OBSERVED INTENSITIES AND F^2_{hk0} VALUES FOR POTASSIUM FORMATE
AMYLOSE

$hk0$	$I_{\text{obs.}}$	F^2_{hk0}	$hk0$	$I_{\text{obs.}}$	F^2_{hk0}
110	20	52	230	2	7
020	29	108	140	6	24
120	0.5	2	240	2	9
220	25	134	430	8	45
130	6	18	540	1	8

and 6. Displacement of the peaks from the computed positions of Patterson $P_w(u,v)$ near $(1/2, 0)$, $(1/2, 1/2)$, and $(0, 1/2)$ may be caused by superposition of amylose-amylose and ion-amylose interactions. This is confirmed by the occurrence of maxima at these positions on the corresponding projection for potassium formate amylose, shown in Figure 7. Observed intensities and F^2 values for the forms contributing to this projection are given in Table VII. Amylose-amylose interactions should be relatively more important in this case, and we assign the large peak at $(1/2, 1/2)$

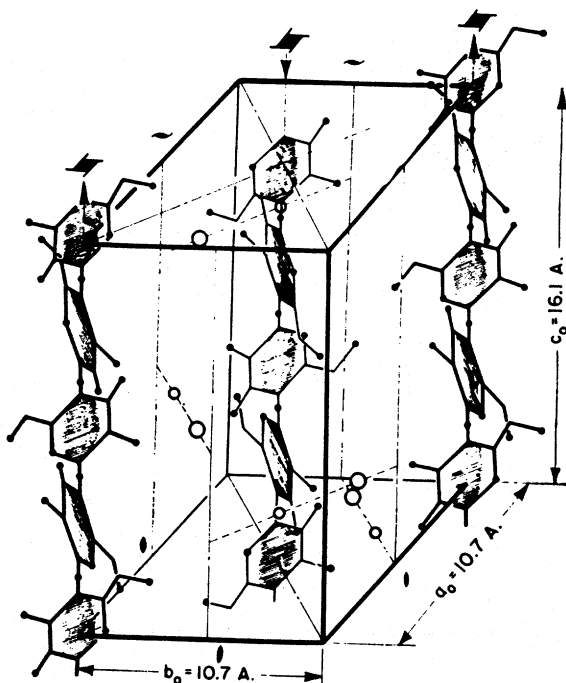


Fig. 8. Structure of potassium bromide amylose. Bromide ions are designated by large open circles, potassium ions by small open circles.

mainly to the interaction between amylose chains in the structure at $(1/2, 0)$ and $(0, 1/2)$. The peaks at $(1/2, 0)$ and $(0, 1/2)$ on the Patterson projec-

tion are ascribed to the combined interactions of K^+-K^+ , and $K^+-\text{amylose}$. Although the peak at $(1/4, 1/4)$ is near the position required by the vector between potassium and formate ions, it may be spurious. Similarly, the extension along the diagonal of the peak at $(0, 0)$ in Figure 7 suggests that the plane of glucose residues may be oriented in this direction in the structure; however, in view of the limited number of F^2 values used in computing these projections, interpretation of such details is hardly justified.

Structure of Tetragonal Addition Compounds. Potassium Bromide Amylose. A structure consistent with the Patterson projections is

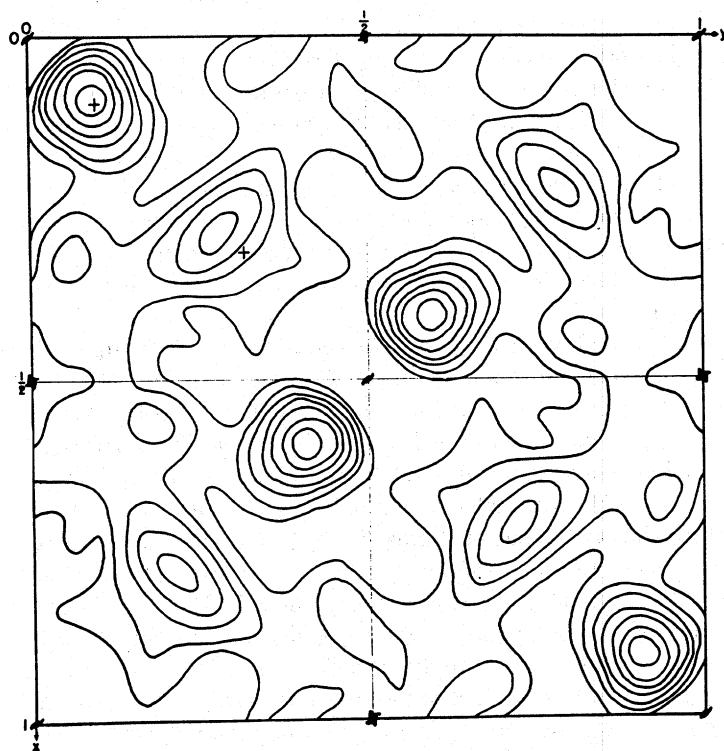


Fig. 9. Fourier projection $\rho_z(x,y)$ for potassium bromide amylose.

represented in Figure 8. This representation is not accurate in detail, for, as noted below, glucoside oxygens of acceptable ring conformations must be displaced from the fourfold screw axes. Also, representation of the glucose ring as a planar hexagon is a simplification. To avoid superposition of atoms, only three chains are shown in Figure 8, and the origin of the unit cell has been placed on a fourfold screw axis, a displacement of $(0, 1/2)$ with respect to the origin selected for the Fourier projection (Figure 9).

Placement of eight glucose residues belonging to two amylose chains in

the cell requires that the chain axes lie on the fourfold screw axes. Aside from the evidence from the Patterson projections, there appears to be no reasonable alternative way of placing eight glucose residues in the cell. Helical or spiral structures having one chain per unit cell do not provide maximum opportunity for hydrogen bonding, and also would be expected to result in hexagonal lateral packing of adjacent chains.

Planes of the glucose residues are oriented to provide an environment of hydroxyl groups about potassium and bromide ions. The near identity of the unit cell dimensions given in Table III shows that the environment about the potassium ion is more important in determining the structure than that about the anion. Furthermore, attempts to replace potassium by sodium, a smaller ion, have resulted in the formation of a different structure, which has a fiber repeat period of 22.6 Å. Neither ammonium bromide nor ammonium iodide forms crystalline addition compounds, indicating that the hydroxyl groups surrounding potassium ions are not tetrahedrally disposed. Ammonium fluoride forms a crystalline addition compound with amylose, but the unit cell is not tetragonal, and the fiber repeat period is 18.9 Å. The high electronegative character and small size of the fluoride ion would also be important structure-determining factors in the latter compound. In the approximate structure represented in Figure 8, adjacent amylose chains are bonded alternately through primary hydroxyl groups and potassium ions.

Fourier Projection for Potassium Bromide Amylose. Although the structure of potassium bromide amylose has no center of symmetry, its projection on (001) is centrosymmetrical, and the phase factors of the structure amplitudes, F_{hk0} , are either +1 or -1. Because of its high scattering power, the bromide ion should determine the phase factors of all F_{hk0} 's with the possible exception of the low-order reflections. F_{hk0} values were calculated for a structure similar to that of Figure 8; in this calculation, it was assumed that the glucose residues have a symmetrical *trans* conformation (model *b*, Figure 10), and that the ring oxygen angle is 100.7°. These computations showed F_{110} to be the only low-order reflection not determined in sign by the bromide ion. Accordingly, the Fourier projection $\rho_z(x,y)$ was evaluated for potassium bromide amylose with all signs except that of F_{110} determined by the contribution of bromide ions at (6/60, 6/60, 0) and positions related by symmetry. In this projection (Fig. 9), not only does a peak readily identifiable as a bromide ion appear at (6/60, 6/60), but another appears at (17/60, 17/60) which has the proper relative height for a potassium ion. The peaks surrounding (1/2, 0) and (0, 1/2) are interpreted to be projections of the amylose chains.

Conformation of the Glucose Residues. Although the general features of the structure presented in Figure 8 appear to be well established, the precise location of carbon and oxygen atoms remains undetermined. Final solution of this problem and, therefore, that of the conformation of the glucose residues, will depend on finding a structure that gives quantitative agreement of calculated and observed intensities. This has not been

accomplished in the present investigation, but some conclusions of interest have been reached in the consideration of Sachse-type conformations of the glucose residues.

The eight Sachse-type ring conformations of alpha-D-glucose are shown in Figure 10. Scattergood and Pacsu³ have constructed Fisher-Hirschfelder models of these conformations and, using the criterion of minimum steric hindrance between substituents, concluded that the symmetrical *trans* conformation, model b of Figure 10, is most nearly correct for alpha-D-glucose. It is difficult to measure interatomic separations on such models; moreover, models can be constructed with only one oxygen angle.

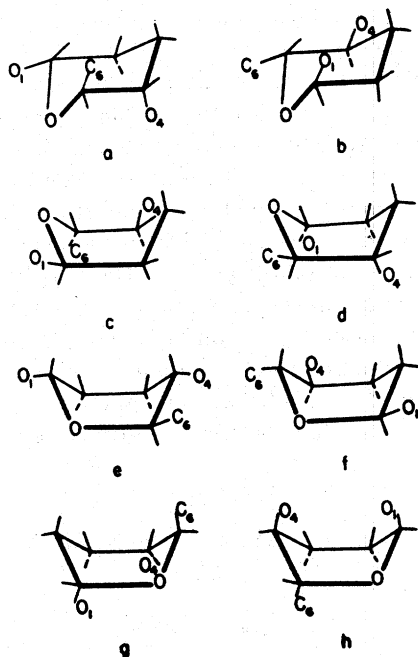


Fig. 10. Ring conformations of alpha-D-glucose.

Since there has been considerable discussion⁴ concerning the ring oxygen angle in cellulose, we constructed accurate metal models in which this angle could be varied. Our measurements are reported in Table VIII. Covalent radii of carbon and oxygen were taken as 0.77 and 0.66 Å., respectively. The C—H distance was taken as 1.09 Å., and all bond angles at carbon atoms were constructed to be $109^{\circ}28'$. Models were constructed to the scale of 2 inches = 1 Å. Comparison with interatomic separations calculated for the symmetrical *trans* configurations indicates that the distances reported in Table III are accurate within ± 0.03 Å. Since the distance between glucoside oxygens O₁ and O₄ must be equal to or greater than 4.03 Å. to satisfy the fiber repeat period of the tetragonal addition compounds, several conformations can be eliminated immediately. These are

the asymmetrical *cis* formations g and h, the symmetrical *cis* conformation d, and, for ring oxygen angles less than 100° , the symmetrical *trans* conformation b. Decision among the remaining conformations is not easily made, but with a requirement of minimum steric obstruction, the symmetrical *trans* conformation b appears to be the most probable. Recent analyses of the structures of glucose⁵ and of the addition compound of sodium bromide with sucrose,⁶ $\text{NaBr} \cdot \text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot 2\text{H}_2\text{O}$, have shown that the glucose ring in these compounds has conformation b.

A further condition which must be satisfied by an acceptable conformation is imposed by the fourfold screw axis. If the glucoside O—O distance for a given conformation exceeds one-fourth the fiber repeat period, the residue must be inclined to the fiber axis. Repetition of this residue by the fourfold screw axis then determines the bond angle at the glucoside oxygens and also the distance between atoms in adjacent residues. The only variables for a given ring conformation are the angular orientation of the residues about the line joining the glucoside oxygens and the sense of the fourfold screw axis. Glucoside oxygen angles and the closest contacts between adjacent residues have been calculated for all conformations listed in Table VIII for which the $\text{O}_1\text{—O}_4$ distance is equal to or greater than 4.03 Å. Although the results of these computations limit the possible orientations of the glucose residue for each conformation, only one conformation is eliminated. This is the symmetrical *trans* conformation b having a ring oxygen angle of $100^\circ 46'$. The glucoside oxygen angle is 99° for residues related by either 4_1 or 4_3 , but the distance between C_6 and C_1 of adjacent residues related by 4_1 is 1.95 Å., and that between C_3 and C_1 of adjacent residues

TABLE VIII
DISTANCE BETWEEN GLUCOSIDE OXYGENS FOR RING CONFORMATIONS OF
FIGURE 10

Conformation	Mode	Ring oxygen angle	$\text{O}_1\text{—O}_4$ distance, Å.	Closest contact, ^a Å.
Sym. <i>trans</i> .	a	90°	4.71	$\text{C}_6\text{—H}_1 = 1.30$
“ “	a	$100^\circ 46'$	4.66	$\text{C}_6\text{—H}_1 = 1.76$
“ “	a	$109^\circ 28'$	4.56	$\text{C}_6\text{—H}_1 = 2.23$
				$\text{H}_6\text{—O}_3 = 1.56$
Sym. <i>trans</i>	b	90°	3.73	$\text{O}_1\text{—H}_5 = 1.30$
“ “	b	$100^\circ 46'$	4.03	$\text{O}_1\text{—H}_5 = 1.76$
“ “	b	$109^\circ 28'$	4.31	$\text{O}_1\text{—H}_5 = 2.22$
Sym. <i>cis</i> .	c	$109^\circ 28'$	5.36	$\text{C}_6\text{—H}_1 = 2.23$
Sym. <i>cis</i>	d	$109^\circ 28'$	3.54	
Asym. <i>cis</i> ^b	e	$116^\circ 20'$	5.43	$\text{H}_1\text{—H}_4 = 1.98$
Asym. <i>cis</i> ^b	f	$116^\circ 20'$	5.35	$\text{H}_5\text{—O}_2 = 1.92$
Asym. <i>cis</i> ^b	g	$116^\circ 20'$	3.60	
Asym. <i>cis</i> ^b	h	$116^\circ 20'$	1.77	

^a Subscripts indicate the carbon atom to which substituent is attached. Carbon atoms are numbered according to the conventional system.

^b Models for the asymmetrical *cis* conformations were constructed with the four atoms in the bed of the ring coplanar.

related by 4_3 is 2.20 Å. Both distances are considerably less than the minimum separation acceptable⁷ for nonbonded carbon atoms.

If conformation b with a ring oxygen angle of $109^\circ 28'$ is accepted on the basis of minimum internal steric hindrance, the results in Table IX indicate that space group P_{4_2} is most probable. Adjacent residues related by the fourfold screw, 4_1 , have considerable steric hindrance between C_6 and C_1 for all orientations of the residues. In contrast, orientations can be found for residues related by 4_3 which are satisfactory with respect to the glucoside oxygen angle and contact distances.

TABLE IX
GLUCOSIDE OXYGEN ANGLE AND CONTACTS BETWEEN ADJACENT RESIDUES^a
RELATED BY FOURFOLD SCREW AXIS

Screw symmetry 4_3				Screw symmetry 4_1	
Rotation of residue about O_1-O_4 direction	Glucoside oxygen angle	Closest contact ^b of adjacent residues, Å. $O_3-O_1^1$ $O_2-O_1^1$ ^c		Glucoside oxygen angle	Closest contact ^b of adjacent residues, Å. $C_6-C_1^1$
0°	122.2°	2.45	3.14	110.8°	2.60
45°	118.9	2.71	3.19	111.3	2.76
90°	104.3	2.33	2.44	120.2	2.59
135°	99.6	1.91	1.64	101.8	2.15
180°	80.4	1.72	1.27	87.3	1.78
225°	77.5	1.82	1.44	68.1	1.51
270°	87.6	1.85	2.20	84.7	1.73
315°	107.4	2.28	2.98	81.5	1.93

^a Glucose residues in symmetrical *trans* conformation of model b, Table VIII, with ring oxygen angle of $190^\circ 28'$.

^b Subscripts indicate the carbon atom to which substituent is attached. Carbon atoms are numbered according to the conventional system. Superscripts indicate that the atom belongs to an adjacent residue related by fourfold screw axis.

^c O_R refers to the oxygen atom contained in the glucose ring.

DISCUSSION

An important result of this structure investigation is that the symmetry elements of the structure require the equivalence of all glucose residues in an amylose chain, or at least of those chains in the crystalline portions of the filaments responsible for the discrete diffraction patterns. X-ray diffraction patterns of amylose,⁸ amylose-alcohol complexes,⁹ and alkali amylose² have shown too few reflections to establish the symmetry elements of the structure, or the asymmetric unit of structure has been a maltose or triose residue. These results, of course, do not mean that the glucose residues in the amylose chains are not equivalent; they mean only that the symmetry elements of the structure do not require that they be equivalent.

Another structural feature of the tetragonal additional compounds that appears to be well established is that the structure is not determined by amylose-amylose contacts but that bonding through potassium ions must occur. This is interpreted to occur through potassium ion-hydroxyl con-

tacts. Bonding of adjacent chains through anion contacts is less important, for the unit cell dimensions are relatively insensitive to the size of the anion in an isomorphous series of addition compounds with potassium salts.

Although the conformation of the glucose residue has not been proved, certain possible conformations have been eliminated. If the conformation found in a single crystal analysis⁶ of glucose is accepted, the ring oxygen angle must be greater than 100° , and the most probable space group is $P_{4,2}$.

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Synopsis

Many salts in aqueous alcohol solution can be exchanged for the alkali in oriented filaments of alkali amylose. If the filaments are held taut during exchange, orientation is retained, and excellent fiber patterns are produced. By this method, compounds of amylose were obtained with the iodide, bromide, formate, acetate, and propionate of potassium and with sodium bromide and ammonium fluoride. In addition, potassium bicarbonate-amylose was formed by the action of carbon dioxide on moist filaments of potassium hydroxide-amylose. Data on the composition of the amylose-potassium salt filaments indicate stoichiometric compound formation between amylose and salt, the ratio of salt molecules to glucose residues being 1:2 for the iodide, bromide, formate, and tetragonal form of the acetate, and 1:1 for the propionate and the orthorhombic form of the acetate. As shown by x-ray diffraction patterns, the potassium salts crystallized in two structures. One, based on a tetragonal unit cell, was common to the iodide, bromide, formate, acetate, and bicarbonate; the other, based on an orthorhombic unit, was the stable structure for the acetate and the propionate. Dimensions of the tetragonal unit cells of air-dried filaments of the iodide and bromide were identical, with $a_0 = 10.7$ and c_0 (fiber axis) = 16.1 Å. Corresponding dimensions for the formate and acetate were 10.8 and 16.1 Å., and those for the bicarbonate were 10.8 and 15.8 Å. The orthorhombic structures of the potassium acetate and propionate addition compounds with amylose appeared to be isomorphous, unit cells having $a_0 = 11.0$, $b_0 = 18.1$, c_0 (fiber axis) = 17.9 Å. for the acetate, and $a_0 = 11.4$, $b_0 = 18.0$, $c_0 = 17.6$ Å. for the propionate. The tetragonal structural modification is of particular interest. Observed diffraction maxima were consistent with the enantiomorphous space groups, $P_{4,2}$ and $P_{4,2}$, and this, together with composition and density data, demands that the amylose chains possess fourfold screw symmetry, with four glucose residues corresponding to the fiber repeat period of 16.1 Å. Successive residues in the amylose chain must, therefore, be identical. This is the first x-ray evidence which has demonstrated the identity of successive residues in the amylose chain.

From a Patterson projection of potassium bromide amylose, the positions of the in-

organic ions were determined. The Fourier projection, $\rho_z(x,y)$, computed from F_{hko} 's, with signs determined by the bromide ion, confirmed the location of the inorganic ions and gave a general outline of the packing of the amylose chains.

Résumé

De nombreux sels en solution hydroalcoolique peuvent être échangés avec l'alcali dans les filaments orientés d'alcali-amylose. Si les filaments sont maintenus tendus durant l'échange, l'orientation est maintenue, et un réseau excellent de fibre est obtenu. Par cette méthode, on a obtenu des dérivés de l'amylose avec les iodures, bromures, formiates, acétate et propionate de potassium et avec le bromure de sodium ainsi qu'avec le fluorure ammonique. En plus, on obtient l'amylose-bicarbonate de potassium par l'action de l'anhydride carbonique sur les filaments humides de l'amylose-hydroxyde de potassium. Les analyses de la composition de l'amylose-sel potassique filamenteux indiquent la formation d'un dérivé stoechiométrique entre l'amidon et le sel, le rapport des molécules de sel aux résidus glucoses étant 1:2 pour les iodure, bromure, formiate et acétate, (forme tétragonale) tandis qu'il est 1:1 pour le propionate et la forme orthorhombique de l'acétate. Ainsi qu'il résulte des diagrammes-X de diffraction, les sels potassiques cristallisent en deux structures distinctes. L'une, basée sur une unité périodique tétragonale, est propre aux iodure, bromure, formiate, acétate et bicarbonate; l'autre, basée sur un motif orthorhombique, est la structure stable pour l'acétate et le propionate. Les dimensions des cellules unitaires tétragonales des filaments séchés à l'air sont identiques dans les cas de l'iodure et du bromure, avec $a_0 = 10.7$ et c_0 (axe de la fibre) = 16.1 Å. Les dimensions correspondantes pour le formiate et l'acétate sont 10.8 et 16.1 Å, et ceux pour le bicarbonate sont 10.8 et 15.8 Å. Les structures orthorhombiques des produits d'addition à l'amylose de l'acétate et du propionate apparaissent être isomorphes, avec des cellules unitaires présentant $a_0 = 11.0$, $b_0 = 18.1$ et c_0 (sens de la fibre) = 17.9 dans le cas de l'acétate, tandis que pour le propionate on a $a_0 = 11.4$, $b_0 = 18.0$ et $c_0 = 17.6$ Å. La structure tétragonale est une modification particulièrement intéressante. Les maxima de diffraction observés correspondent aux groupes énantiomorphes P_{4121} et P_{421} , et ceci, ajouté aux résultats des compositions analytiques ainsi que des densités, supposent que les chaînes d'amylose possèdent une symétrie hélicoïdale quadruple, avec un motif périodique constitué de quatre restes glucosés comportant 16.1 Å. Les parties successives dans les chaînes d'amylose doivent donc être identiques. Ceci constitue la première preuve au moyen des rayons-X de l'identité des parties successives dans la chaîne de l'amylose. Au moyen d'une projection suivant Patterson de l'amylose-bromure de potassium, la position des ions inorganiques a été déterminée. Une projection de Fourier, $\rho_z(x,y)$ obtenues aux dépens des F_{hko} , avec des signes déterminés par les ions bromures, confirment la localisation des ions inorganiques et donnent une allure générale du tassement des chaînes amylosiques.

Zusammenfassung

Viele Salze können in wässriger Alkohollösung gegen das Alkali in orientierten Fasern von Alkali-Amylose ausgetauscht werden. Wenn die Fasern während des Austausches straff gehalten werden, wird die Orientierung beibehalten, und es werden ausgezeichnete Faserdiagramme erhalten. Mit dieser Methode wurden Verbindungen von Amylose mit Kaliumjodid, -bromid, -formiat, -acetat und -propionat und mit Natriumbromid und Ammoniumfluorid erhalten. Ausserdem wurde Kaliumbikarbonat-Amylose durch Einwirkung von Kohlendioxyd auf feuchte Fasern von Kaliumhydroxyd-Amylose gebildet. Daten über die Zusammensetzung der Amylose-Kaliumsalz-Fasern zeigen die Bildung einer stöchiometrischen Verbindung zwischen Amylose und Salz, wobei das Verhältnis der Salz-moleküle zu den Glukoseresten für das Jodid, Bromid, Formiat und die tetragonale Form des Acetates 1:2 ist, und für das Propionat und die orthorhombische Form des Acetates 1:1. Wie durch Röntgendiffraktionsdiagramme gezeigt wird, kristallisieren die Kaliumsalze in zwei Modifikationen. Die eine, die auf eine tetragonale

Einheitszelle aufgebaut war, war dem Jodid, Bromid, Formiat, Acetat und Bikarbonat eigen; die andere, die auf die orthorhombische Einheit gestützt war, war die stabile Modifikation für das Acetat und das Propionat. Die Dimensionen der tetragonalen Einheitszellen der luftgetrockneten Fasern des Jodids und Bromids waren identisch, mit $a_0 = 10.7$ und c_0 (Faserachse) $= 16.1$ Å. Die entsprechenden Dimensionen für das Formiat und das Acetat waren 10.8 und 16.1 Å., und die für Bikarbonat waren 10.8 und 15.8 Å. Es wurde gefunden, dass die orthorhombischen Modifikationen der Kaliumacetat und -propionat-Additionsverbindungen mit Amylose isomorph waren, wobei ihre Einheitszellen für das Acetat $a_0 = 11.0$, $b_0 = 18.1$, und c_0 (Faserachse) $= 17.9$ Å. haben, und für das Propionat $a_0 = 11.4$, $b_0 = 18.0$ und $c_0 = 17.6$ Å. Die tetragonale Strukturänderung ist besonders interessant. Die beobachteten Diffraktionsmaxima stimmten mit den enanthiomorphen Raumgruppen $P_{4_12_1}$ und $P_{4_22_1}$ überein, und dies, wie auch die Zusammensetzungs- und Dichtedaten fordern, dass die Amyloseketten vierfache Schraubensymmetrie haben, wobei vier Glukosereste der Faser-Wiederholungsperiode von 16.1 Å. entsprechen. Aufeinanderfolgende Glukosereste in der Amylosekette müssen daher identisch sein. Dies ist der erste Beweis durch Röntgenstrahlen, der die Identität von aufeinanderfolgenden Resten in der Amylosekette zeigt. Aus einer Patterson-Projektion von Kaliumbromid-Amylose wurde die Stellung der anorganischen Ionen bestimmt. Die Fourier-Projektion, $\rho_z(x, y)$, berechnet aus F_{hk0} 's, dessen Zeichen durch das Bromidion bestimmt wurde, bestätigte die Stellung der anorganischen Ionen und gab eine allgemeine Erklärung der Packung der Amyloseketten.